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IDENTIFICATION OF BUTTERNUT WINTER SQUASH CAROTENOIDS AND ITS UTILIZATIONS AS A NATURAL ANTIOXIDANT AND COLORANTS IN SOME FOODS

E. M. Rizk and Gamila Y. Attia

Food Technology Res. Institute, Agric. Res. Center - Giza - Egypt

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ABSTRACT: ***The study structure of carotenoid pigments extracted from butternut winter squash by using thin layer chromatography (TLC) and High Performance Liquid Chromatography (HPLC) and the relation between adding of these caroteniod pigments in some processed food utilization as antioxidants and natural coloring materials. The obtained data showed that, the best carriers of butternut winter squash carotenoid was lactose followed by dextrin. Five peaks were identified by TLC and HPLC in case of butternut winter squash carotenoids namely lutein, cryptoxanthin, γ carotene, α carotene and β carotene. On the other hand, the sunflower oil contained 300 to 500 ppm butternut winter squash carotenoid recorded high stability (higher induction period) than sunflower oil contain 200 ppm synthetic antioxidant (BHT) according to he Rancimat test. Addition of 0.30 to 0.40 butternut winter squash carotenoid pigments (as a natural colorant) improved the color, taste, general acceptability of drops compared to other tested samples.***

Key words: ***Natural yellow pigments, carotenoids, butternut winter squash, natural antioxidant, drops.***

INTRODUCTION

Butternut winter squash *Cucurbita moscata* is a type of winter squash. It has a sweet, nutty taste that similar to pumpkin. Butternut winter squash shaped like a large pear. It has yellow skin and orange fleshy pulp after ripening. It turns increasingly deep orange and becomes sweeter. It is a good source of fiber, vitamin C, manganese magnesium and potassium. It is also an excellent source of carotenoids and its derivative i,e. vitamin A.

The butternut winter squash is considered the main source of caroteniods which is responsible for orange and yellow colors. These colors are very important in the acceptability of such products. Besides, several components present in these natural colors "caroteniods" are also the precursor of vitamin A which is essential in human nutrition.

β carotene has been used many years as a health food product under the claim "antioxidant". Many epidemiological and oenological studies suggest

that humans feed on a diet high in caroteniod – rich vegetables and fruits, who maintain higher than average levels of serum carotenoids, have a lower incidence of several type of cancer and cardiovascular disease (Albances *et al* 1996) and (El – Sherefa 2004).

The intake of foods rich in carotenoids appears to be associated with optimal health, and a reduction in the risk of cancer, cardiovascular disease, macular degeneration and cataract formation. Hydrocarbon carotenoids such as alpha, beta carotenes and lycopene may reduce the risk of cancer and heart disease, whereas oxygenated carotenoids, such as lutein and zeaxanthin, may be important to the protection of the eye. (Yeum 1996).

Consumer interest in β carotene has been developed as the media attention is focusing on the protective role of β carotene. The evidence that β carotene may act as a cancer preventive agent derives from a number of epidemiological studies which showed that high intake of β carotene correlates to a later observed lower incidence of certain type of cancer (*Charleux, 1991*). It has been suggested that, β carotene may have a role independent of its vitamin A activity. β carotene in addition to its provitamin A activity appears to play a role in supporting the body's defense mechanism against cancer and chronic degenerative disease.

Caroteniods have been used as food colors for countries. Color of carotenoids, together with beneficial properties such as vitamin A precursor and anti oxidant; have led to their wide application in the food industry. Preparations to apply them in oily or aqueous media have been produced including emulsions, colloidal suspensions and complexes with proteins. These preparations have found applications as a colorant in many food such as margarine, butter, fruit juices and beverages, canned soups, dairy and related products, deserts and mixes, preserves and syrups, sugar and flour confectionary, salad dressing, egg products, among other and interestingly other important areas of application of carotenoids have been emerged (Delgado Vargas *et al* 2000 and Sayed 2008).

The synthetic food colorants have led to the prohibition of the use of some of it in food due to the discovery of possible toxicity substances in them. On top of that, the less stringent tests necessary for the use of natural colorants and the increase in the demand for natural ingredients by increasingly health conscious consumers have led food manufactures to take another look into the use of natural food colorants. This has also resulted in a proliferation of interest in the development of natural food colorants, as can be seen from the vast number of patents field in recent years (Francis, 1987 and Sayed 2008).

The use of synthetic antioxidant such as butylated hydroxy toluene (BHT) and butylated hydroxyl anisol (BHA) cause harmful effects on humans (Farag *et al* 2003). In this connection, and easily decompose at high temperature In addition (Change *et al* 1977) showed that BHT and BHA are quite volatile,

these synthetic antioxidants are not effective in preventing the development of initial off flavor.

The use of natural antioxidants is highly desirable to replace the synthetic antioxidants. In this respect, the extracts of several plants have been reported to possess wide degrees of antioxidant activities (Kim *et al* 1994, Rizk *et al* 2008 and Azouz *et al* 2007).

The aims of this study were identification and characterization of carotenoids from butternut winter squash and its utilization as natural anti oxidant isolated food colorants.

MATERIALS AND METHODS

Samples:

Butternut winter squash *Cucurbita moscata* were brought from Kaha Agriculture Research Station, Horticulture Research Institute in March 2007.

Chemical and Reagents:

The solvents used for spectral and HPLC analysis were of HPLC grade and all others solvents were of ACS grade.

Refined sunflower oil free from antioxidants was obtained from Arma Food Industries, 10th of Ramadan.

Synthetic antioxidant, namely butylated hydroxyl toluene (BHT) was purchased from Sigma Chemical Co. St Lewis, U.S.A.

Lactose, dextrin and soluble starch ascorbic acid were obtained from ADWIC (El-Nasser Pharmaceutical Chemicals Company).

Wheat flour 72% extraction was purchased from local market.

Chemical analysis:

Extraction and concentration of cartoenoids.

The butternut winter squash sample was extracted and concentration by the method reported by Nilzu and Rodriguez-Amaya (2005) with cold acetone in the presence 0.1% ascorbic acid using a mortar and pestle. Extraction and filtration on Buchner was repeated until the residue was devoid of color (about 3 times), the total amount of acetone used being 300ml. the carotenoids partitioned to 100ml peteroleum ether and saponified overnight with an equal-volume of 10% KOH in methanol. After washing, the carotenoid solution was concentrated in rotary vacuum evaporator at 40°C.

The concentrated yellow pigments of butternut winter squash were adsorbed in different ratio of solid matrixes i.e. (Lactose, soluble starch, wheat flour and dextrin) up to 6: 1 pigment matrix and dried in oven at 40^{o}c for 24hr.

Determination of total carotenoids:

Total caroteniods were determined by the method of (Reddy and Sistrunk 1980). The O.D was measured at 440 nm and compared to β carotene standard curve.

Identification of carotenoid extracted from butternut winter squash.

Thin layer chromatography (TLC) analysis:

TLC was applied suing silica gel GF. 245 for identification of butternut winter squash carotenoid by method reported by (Eder 1996). Extracted carotenoid was dissolved in a small amount of acetone and spotting in TLC. The plate was developed with solvent system methylene chloride, ethylacetate (4:1). Then dried at room temperature. For visualization of color spots P-anisaldehyde was used and RF value was calculated.

High Performance Liquid Chromatography (HPLC) analysis:

The carotenoids of butternut winter squash were identified by Knwuer HPLC pump 64 according to the method reported by Gaylek *et al* (1987) using octadecyl silence C 18, 3.9 X 150 mm. for both HPLC column two solvents were used for elution: (1) methanol (2) ethylacetate. The flow rat was 1-8ml/min and absorbance was measured at 475 mm.

Antioxidant activity:

Determination of induction period with Rancimat method.

Rancimat 679 (Metrom Ltd 9100 Herisau, Switzerland) was used for the determination of oxidative stability of sun flower oil. The induction time was automatically determined, i.e. the time from the start of the experiment to the intersection point (Mendez *et al* – 1996).

Preparation of sweet drops:

Drops were prepared by mixing sucrose (242.4g), corn syrup (129.5g) and citric and (0.75g) then heated upto 157^{o}C. Quickly, it was cooled to 110^{o}C and added (1.05g) flavoring agent ad natural yellow color (0 -1 to 0.5% from carotenoid pigment extracted from butternut winter squash). These contents should be mixed very well then put in forming blocks for solidifying, and then packed in special foil (Sayed 2008).

Sensory evaluation of drops:

Ten panelists were asked to evaluate color, taste and overall acceptability of drops. Sensory evolution was carried out according to the procedures described by (Reitmeier and Nonnecke 1991).

Statistical analysis:

Means of data obtained for sensory evaluation the samples were evaluated using Duncan's multiple range test to identify significant differences at the 0.05 probability ($p \leq 0.05$) using the statistical Analysis system (SAS) SAS Institute Ince. (1999).

RESULTS AND DISCUSSION

Carotenoids content of butternut winter squash and its distribution within selected carrier.

It is well know that within the mechanism of adsorption spraying of carotenoids should realize the separation of pigments in droplets in order to prevent their sticking together. This could be usually achieved by using a suitable powder or liquid medium to catch the particles. The powder phase will adhere to the surfaces of the pigment particles while; liquid will form a film around them (Counsel 1980 and Rizk and Tolba 2002). With this view in mind the average yield of extracted yellow colorants determined as cartoenoids is given in Table (1). The concentration of the extracted carotenoids was 3.4mg / 100gfresh butternut winter squash sample. From the same table it could be concluded that, a total 100g of mixing caroteniod with coated carrier (6:1) i.e. lactose, soluble starch, wheat flour and dextrin represented (in the presence 0.1 %ascorbic acid) 10.42, 3.46, 2.18 and 6.93g carotenoids extracted from butternut winter squash respectively. This means that, lactose was the most effective adsorbent coated carrier material for yellow colorant extracted from butternut winter squash followed by dextrin, soluble starch and wheat flour respectively. These results are confirmed by (Rizk and Tolba 2002).

Table (1); Distribution pattern of carotenoids extracted from butternut winter squash with in selected carrier

Rate of carotenoids to carrier g /100 g	Lactose	Soluble starch	Wheat flour	Dextrin
1 : 1	0.56	0.32	0.29	0.39
2 : 1	1.52	0.63	0.58	0.61
3 :1	3.27	0.86	0.73	1.02
4 : 1	5.34	1.23	0.97	2.54
5 :1	8.32	2.32	1.42	4.26
6 : 1	10.42	3.46	2.18	6.93

*** Total carotenoid of butternut winter squash was 3.4 mg/100g**

The reason of adding ascorbic acid during dispersion of extracted carotenoid (from butternut winter squash) on the applied carrier is based on its higher capability of absorbing the oxygen surrounding the extracted coloring substances. In other words, ascorbic could be oxidized more rapidly than the extracted coloring substances, patterns which lead to more stabilization of the tested pigment, (Counsel 1980 and Rizk *et al* 2008).

Separation and identification of butternut winter squash carotenoide by using TLC and HPLC

A typical HPLC data given in Table (2) summarized the carotenoid extracted from butternut winter squash. Carotenoid were separated based on their functional groups into four fractions by thin layer chromatography (TLC) on silica gel. Idea about the TLC was mainly used for preliminary examination to give an indication of the number and variety of carotenoid components present in the extract and to help in the selection of a suitable separation and purification procedure for the given mixture (Eder 1996 and Rizk and Tolba 2002).

Table (2): TLC and HPLC patterns of carotenoid pigments extracted from butternut winter squash

TLC	HPLC data		Retention time (min)	Identified fraction
RF X 100	Peak No.	Concenetration %		
88	1	33%	25	Lutein
92	2	8%	36	Cryptoxanthin
56	3	13%	30	γ carotene
63	4	20%	32	α Carotene
76	5	26%	39	β carotene

The calculated RF values for carotenoid fractions of butternut winter squash were 88, 92 56, 63 and 76 respectively. On the other hand, five peaks were identified by using HPLC chromatogam namely lutien, cryptoxanthin, γ carotene, α carotene and β carotene respectively.

The concentration of these fractions was 33% lutein, 26% β carotene 20% α carotene,13% γ carotene and 8% cryptoxanthin.

Antioxidants activity of butternut winter squash carotenoid

Antioxidant activity of butternut winter squash carotenoids. The effect of different concentration (200, 300 , 400, 500 and 600 ppm) of the extracted carotenoid from butternut winter squash on the stability of sunflower oil compared to the sunflower oil contain 200 ppm BHT and sunflower oil without any added are given in Table (3). Stability of sunflower oil was measured at 110^{o}C by Rancimate method.

The induction period was increased gradually as the concentration of carotenoid butternut winter squash from 200 to 600ppm. The induction

period of sunflower oil containing 200ppm BHT and sunflower oil without any added were 4.15 and 2.65h respectively. On the other hand, the induction period of sunflower oil containing 200,300,400,500 and 600ppm from carotenoid extracted butternut winter squash were 4.30, 7.90, 8.70, 9.60 and 10.30 h respectively.

Table (3) The effect of different concentration of carotenoid extracted from butternut winter squash and BHT on the stability of sunflower oil

Treatments	Induction period (h)
Sunflower oil only	2.65
Sunflower oil + 200ppm BHT	4.15
Sunflower oil + 200ppm carotenoid extract	4.30
Sunflower oil + 300 ppm carotenoid extract	7.90
Sunflower oil + 400 pm carotenoid extract	8.70
Sunflower oil + 500 pm carotenoid extract	9.60
Sunflower oil + 600 pm carotenoid extract	10.30

From the aforementioned results, it could be noticed that, the sunflower oil containing 300 to 500 ppm carotenoid from butternut winter squash recorded high stability (higher induction period in Rancimat test) than sun flower oil contain 200 ppm synthetic antioxidant (BHT).

These results are agreement wit (Nilson *et al* 1999, Azouz *et al* 1999, Azouz *et a*l 2007 and Rizk *et al* 2008).

These compounds are considered to be beneficial to health act as since its antioxidants in the body inhibiting lipid by pro oxidation scavenging, free radicals and displaying anti mutagenic properties. These results suggest that the carotenoids extracted from butternut winter squash possess antioxidant properties could be used alternatives natural antioxidants with wide food applications.

Sensory evaluation of prepared drops:

Sensory properties of drops prepared by adding different levels of carotenoid extracted from butternut winter squash in the range of 0.10 to 0.50% are given in Table (4). On using Duncan's multiple range test, it could be concluded that, in the presence 0.30% butternut squash carotenoid pigment a superior color of the tested samples. On contrary, the inferior color was recorded in samples prepared with 0.10% butternut winter squash caroteniod.

Table (4) Average values of various sensory parameters of the tested drops with different level of butternut winter squash carotenoid pigment

Variable	Color	Taste	Overall acceptability
Drops +			
0.10% pigments	4.6^{e}	5.2^{e}	4.2e
0.20%	6.3^{d}	6.0^{d}	6.0^{d}
0.30	9.8^{a}	9.5^{a}	9.6^{a}
0.40	9.0^{ab}	9.3^{a}	9.5^{a}
0.50	7.9^{c}	8.7^{b}	8.3^{b}

From the aforementioned results, it could be noticed that drops prepared with of 0.30 and 0.40% of butternut winter squash carotenoid pigment have an improved color, taste and overall acceptability, while more than 0.40 or less than 0.20% butternut winter squash carotenoid pigment led to unacceptable drops.

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التعرف على كاروتينويدات الكوسة الشتوي واستخدامها كمضادات أكسدة وملونات طبيعية فى بعض الأغذية

عفت مهدى رزق ، جميلة يوسف عطية

معهد بحوث تكنولوجيا الأغذية . مركز البحوث الزراعية . الجيزة . مصر

المخلص العربي

تم دراسة تركيب الكاروتينويدات المستخلصة من الكوسة الشتوي والتعرف على مكونات هذا المستخلص باستخدام طريقتى TLC و HPLC وأيضاً تم دراسة تأثير إضافة الكاروتينويدات المستخلصة إلى زيت عباد الشمس لمعرفة نشاطها كمادة طبيعية مضادة للأكسدة وأيضاً استخدمت كمادة ملونة طبيعية فى الحلوى.

وأظهرت النتائج المتحصل عليها ما يلي:

١- أفضل مادة لتحميل الكاروتينويدات (اللون الأصفر) المستخلصة هو اللاكتوز ثم الدكسترين حيث كان معدل التحميل ١٠.٤٢، ٦.٩٣ جرام كاروتينويد لكل ١٠٠ جرام مادة تحميل على التوالى.

٢- وباستخدام طرق التحليل الكروماتوجرافي أمكن التعرف على وجود خمسة مركبات بالمستخلص وهى الليوتين ٣٣% . الكربثوكزانثين ٨%ـ جاما كاروتين ١٣%ـ الألفاكاروتين ٢٠%. البيتاكاورتين ٢٦%.

٣- وعلى الجانب الآخر أظهرت النتائج أن إضافة ٣٠٠ إلى ٥٠٠ جزء في المليون من الكاروتينويدات المستخلصة إلى زيت عباد الشمس أعطت فترة ثبات أعلى من الزيت المحتوى على ٢٠٠ جزء في المليون من مضاد الأكسدة الصناعي HBT.

٤- وكذلك أوضحت النتائج أن إضافة ٠.٣ إلى ٠.٤% من الكاروتينويدات المستخلصة (اللون الأصفر) أدى إلى تحسين الخواص الحسية للحلوى المصنعة Drops من قبل المحكمين.